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Angiotensin-converting enzyme 2: a new target for neurogenic hypertension

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Overactivity of the renin–angiotensin system (RAS) is involved in the pathogenesis of hypertension, and an overactive brain RAS has been highlighted in several genetic and experimental models. Until now, angiotensin II (Ang II) was thought to be the main effector of this system, and the angiotensin-converting enzyme (ACE)–Ang II–Ang II type 1 receptor axis was the main target for antihypertensive therapies. A new member of the RAS, ACE2 (angiotensin-converting enzyme type 2), has been identified in organs and tissues related to cardiovascular function (e.g. heart, kidney and blood vessels) and appears to be part of a counter-regulatory pathway to buffer the excess of Ang II. We recently identified the ACE2 protein in brain regions involved in the central regulation of blood pressure and showed that it regulates, and is regulated by, other components of the RAS. Here, we present evidence for the involvement of brain ACE2 in the central regulation of blood pressure, autonomic and cardiac function. We show that lack of ACE2 is deleterious for the central regulation of blood pressure and that brain *ACE2* gene therapy can restore baroreflex and autonomic functions and prevent the development of hypertension. Additionally, and independently of a reduction in Ang II levels, we will highlight some of the mechanisms responsible for the beneficial effects of central ACE2 in cardiovascular function.

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Hypertension affects about 74 million people in the United States aged ≥20 years. However, only 25% of these patients have their blood pressure under control. One of the reasons for this poor record is that most cases of hypertension (about 90–95%) have an unknown cause and are therefore termed primary hypertension or essential hypertension. The sympathetic nervous system exerts a fundamental role in the homeostatic control of blood pressure (Grassi & Mancia, 2004). Studies have shown that several haemodynamic changes in hypertension, such as elevated cardiac output and heart rate, as well as alteration of vascular resistance, can be neurogenic (Julius & Majahalme, 2000). Indeed, neurogenic hypertension is associated with a rise in sympathetic outflow and, often, an inhibition of parasympathetic drive, resulting in the increase of cardiac output and peripheral vascular resistance. In addition, plasma noradrenaline level, a marker of sympathetic activation, is elevated in essential hypertensive patients compared with age-matched normotensive control subjects (Goldstein, 1983).

The renin–angiotensin system

The renin–angiotensin system (RAS) has been shown to play an important role in blood pressure regulation (Bader & Ganten, 2008). The classical view of the RAS (Paul *et al.* 2006), also called the endocrine RAS, is that angiotensinogen is released from the liver and cleaved in the circulation by renin, an enzyme secreted from the juxtaglomerular apparatus of the kidney, to form the decapeptide, angiotensin I (Ang I). Angiotensin I is then transformed into the octapeptide, angiotensin II (Ang II), by angiotensin-converting enzyme (ACE), a membrane-bound metalloproteinase, which is

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predominantly expressed in high concentrations on the surface of endothelial cells in the pulmonary circulation. Angiotensin II, as the primary modulator in this system, then acts on specific receptors, the type 1 receptors, leading to vasoconstriction. In recent years, the discovery of these RAS components in various tissues has led to the concept of a 'local' or 'tissue' RAS (Lavoie & Sigmund, 2003). This concept was based on the discovery of RAS components in 'unlikely' places, such as the 'kidney enzyme', renin, being found in the brain, and instances in which the endocrine actions of the system could not explain the findings, for example the beneficial effects of ACE inhibitors in patients with normal plasma Ang II levels. It is now established that a local RAS is present in various tissues throughout the body, regulating local organ function and interacting with the endocrine RAS as well as with the RAS in other tissues.

The brain RAS

Components of the RAS have been identified in all brain nuclei involved in the central regulation of blood pressure, including the subfornical organ (SFO), paraventricular nucleus (PVN), rostral ventrolateral medulla (RVLM), area posterma (AP) and nucleus of the tractus solitarius (NTS; Davisson, 2003). In addition to Ang II, other angiotensin peptides have also been identified in the brain, such as angiotensin-(1–7) (Ang-(1–7); Schiavone *et al.* 1988), angiotensin IV (Ang IV; Faure *et al.* 2008; Yang *et al.* 2008) and, recently, angiotensin-(1–12) (Ang-(1–12); Nagata *et al.* 2006). Angiotensin-(1–7) has properties opposite to those of Ang II. For example, it stimulates nitric oxide (NO) release, improves baroreflex function and promotes vasodilatation (Sakima *et al.* 2005; Sampaio *et al.* 2007), whereas Ang II impairs these mechanisms. Most of the previously mentioned nuclei are inside the blood–brain barrier and are therefore protected from systemic neuromediators. However, some of them, called circumventricular organs (CVO), such as the SFO and AP, lack a blood–brain barrier and, as a result, constitute 'opened windows' to the brain for small peptides, such as Ang II (Johnson & Thunhorst, 1997). Indeed, in addition to locally generated Ang II in the brain, blood-borne Ang II can reach the brain via the CVO and interact with angiotensin receptors located in these areas to exert central effects in addition to its peripheral effects (Lazartigues *et al.* 2007; Xia *et al.* 2009).

Role of ACE2 in the brain

A decade ago, a new member of the RAS was discovered and, as the first homologue of ACE, was named ACE2. Unlike ACE, ACE2 is a mono-carboxypeptidase and shares 42% homology with ACE (Tipnis *et al.* 2000). While ACE generates Ang II from the degradation of Ang I, ACE2 is able to cleave Ang II and produce the vasodilating peptide, Ang-(1–7) (Vickers *et al.* 2002). Studies have shown that peripheral ACE2 is able to reduce cardiac hypertrophy and prevent the development of hypertension in various animal models (Diez-Freire *et al.* 2006; Rentzsch *et al.* 2007). In the CNS, Yamazato *et al.* (2007) showed that ACE2 overexpression in the RVLM could reduce the elevated blood pressure in spontaneously hypertensive rats (SHR).

Focusing on one of the CVO, we observed that brain-targeted ACE2 overexpression in the SFO reduces the acute Ang II-mediated pressor and drinking responses (Feng *et al.* 2008). In addition to the obvious reduction of Ang II levels resulting from ACE2 overexpression, we noticed that these responses were also associated with the downregulation of AT_1 receptor expression at both the mRNA and the protein levels. These data suggest that adenovirus-mediated ACE2 expression definitely plays a regulatory role in counter-balancing the effects of Ang II, but also may regulate AT_1 receptor expression. However, owing to the short-term expression and the low efficiency of the virus vectors, these acute studies could not address the long-term effects of ACE2 expression and dissect these mechanisms in detail.

To further investigate the role of ACE2 in the CNS, we generated a new transgenic mouse model (syn-hACE2), in which the expression of the human *ACE2* gene is driven by a synapsin promoter, allowing its expression in every neuron of the brain. The syn-hACE2 transgenic mice exhibit normal baseline cardiac haemodynamic parameters, with blood pressure and heart rate in the same range as their control non-transgenic littermates (NT). Similarly, these transgenic mice have unaltered baseline spontaneous baroreflex sensitivity and autonomic function. A more important question related to the phenotype of these mice in the face of a hypertensive challenge. To test the role of central ACE2 in the development of high blood pressure, we used the Ang II 'slow pressor dose' model via an osmotic pump ($600\ ng\ kg^{-1}\ min^{-1}$), which has been shown to result in neurogenic hypertension (Zimmerman *et al.* 2002). Interestingly, our data show that ACE2 overexpression in the brain blunted the development of low-dose Ang II-induced neurogenic hypertension in syn-hACE2 transgenic mice, as well as the associated increase in water intake. In parallel to these reductions in blood pressure and water intake, baroreflex sensitivity and parasympathetic tone were preserved from the inhibitory effects of Ang II infusion, while, paradoxically, sympathetic outflow did not appear to be significantly reduced. Most importantly, co-infusion of Ang II with the Ang-(1–7) antagonist, D-Ala^7-Ang-(1–7), totally reversed the blood pressure-lowering effects of ACE2, suggesting that Ang-(1–7) plays a pivotal role in the prevention of hypertension in this model.

This protective role of ACE2 was also tested in a genetic model of hypertension, the R^+A^+ mouse, developed by Dr Curt D. Sigmund at The University of Iowa. These mice overexpress both human renin and angiotensinogen genes throughout their body and are chronically hypertensive (Merrill *et al.* 1996). Breeding these mice with the syn-hACE2 model allowed us to generate a triple-transgenic (SARA) mouse, with chronic elevation of Ang II in the brain and the periphery and overexpression of ACE2 in the CNS. Interestingly, the enhanced water intake was prevented and autonomic function improved in the SARA mice. Moreover, hypertension was signifiantly reduced, confirming the potential of ACE2 in the buffering of an overactive RAS (Xia *et al.* 2009).

To gain insight into the signalling pathways and molecular mechanisms that lead to ACE2-mediated reduction of hypertension development, we focused on the regulation of nitric oxide synthase (NOS) expression. Indeed, as a direct product of NOS, NO release has been shown to reduce sympathetic activity in the central nervous system (Sakai *et al.* 2005) and its release is known to be enhanced following activation of the Ang-(1–7) receptor (Sampaio *et al.* 2007). We hypothesized that ACE2 overexpression would lead to an increase in Ang-(1–7)-mediated NO release and therefore modulate the blood pressure and cardiovascular function. Using the syn-hACE2 transgenic mouse model, we examined the expression of the neuronal NOS (nNOS) and endothelial NOS (eNOS), as well as the phosphorylated form of this protein (Ser^{1177}-phosphorylated-eNOS). We observed that the expression of nNOS, eNOS and Ser^{1177}-phosphorylated-eNOS were upregulated in syn-hACE2 mice throughout the brain, including cardiovascular regions as well as non-cardiovascular regions, such as cortex, telencephalon and pons–midbrain (Fig. 1). These data suggest that ACE2 overexpression upregulates the expression and phosphorylation of constitutive NOS isoforms, which may have contributed to the blunting of hypertension through an enhanced release of NO in the CNS.

As previously mentioned above ('*Role of ACE2 in the brain*'), we found that adenovirus-mediated ACE2 overexpression was able to downregulate AT_1 receptors in the SFO. Since the Ang-(1–7) receptor (Mas) and Ang II type 2 (AT_2) receptors have been reported to mediate NOS activation and NO release (Sosa-Canache *et al.* 2000; Xu *et al.* 2008), we investigated whether ACE2 overexpression would modify Mas and AT_2 receptor expression in the CNS. Using immunohistochemistry, we observed that ACE2 was able to modulate the expression of these receptors and eventually increase the AT_2/AT_1 and Mas/AT_1 ratios in the SFO (Table 1). While Ang II infusion resulted in a dramatic reduction of these ratios in control and transgenic mice, probably resulting from the upregulation of AT_1 receptors, the syn-hACE2 mice were less affected and therefore protected from the deleterious effects of the vasopressor peptide. However, blockade of the Ang-(1–7) receptor reversed this protective effect, suggesting that Ang-(1–7) plays a critical role in the modulation not

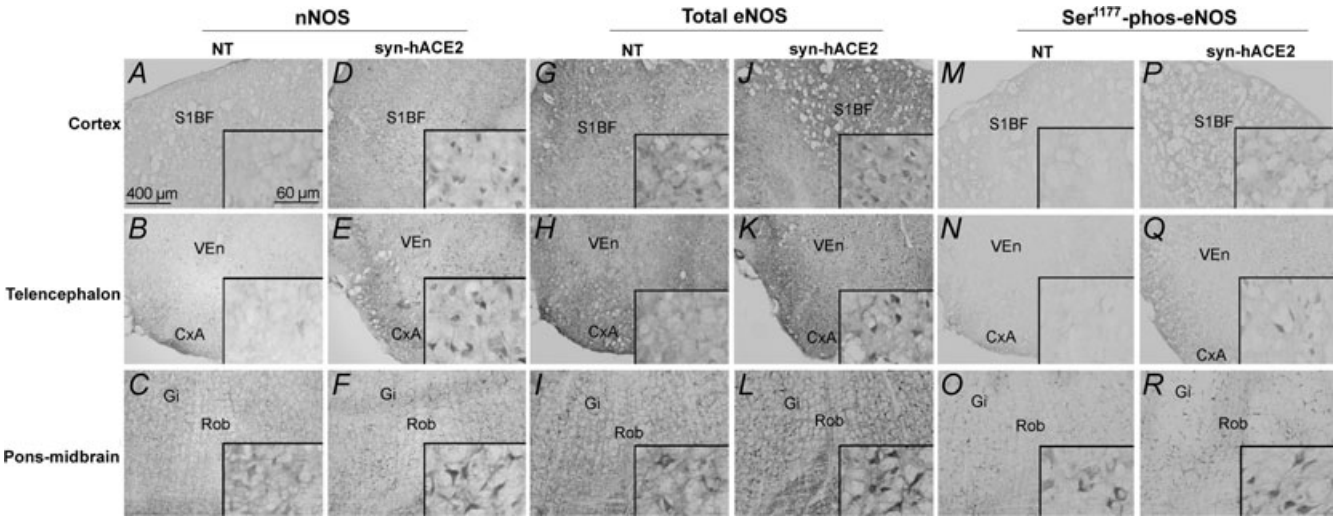

Figure 1. Nitric oxide synthase (NOS) expression in the brain
Immunohistochemistry for neuronal NOS (nNOS), endothelial NOS (eNOS) and Ser^{1177}-phosphorylated-eNOS was visualized by diaminobenzidine. Baseline levels of nNOS, eNOS and Ser^{1177}-phosphorylated-eNOS were all significantly elevated throughout the brain in syn-hACE2 mice in cortex (*D*, *J* and *P*), telencephalon (*E*, *K* and *Q*) and pons–midbrain (*F*, *L* and *R*) compared with control mice in cortex (*A*, *G* and *M*), telencephalon (*B*, *H* and *N*) and pons–midbrain (*C*, *I* and *O*). Abbreviations: NT, non-transgenic; syn-hACE2, human *ACE2* transgenic mice; S1BF, the primary somatosensory cortex-barrel field; VEn, ventral endopiriform nucleus; CxA, cortex–amygdala transition zone; GI, gigantocellular reticular nucleus; and Rob, raphe obscurus nucleus.

Table 1. Ratio of AT_2/AT_1 and $Mas1/AT_1$ receptors in subfornical organ of syn-hACE2 transgenic mice (SA) and non-transgenic mice (NT). NT were the littermates of SA mice

	AT_2/AT_1 ratio	$Mas1/AT_1$ ratio
NT + saline	1.0 ± 0.14	1.0 ± 0.17
SA + saline	1.89 ± 0.04*	1.86 ± 0.22*
NT + Ang II	0.09 ± 0.06†	0.89 ± 0.14
SA + Ang II	0.53 ± 0.08†	1.07 ± 0.20
SA + Ang II + D-Ala^7-Ang-(1–7)	0.29 ± 0.08‡	0.57 ± 0.12

Data represent the relative receptor density normalized to NT + saline. Saline, Ang II, D-Ala^7-Ang-(1–7) were infused by osmotic pump. Values are expressed as means ± S.E.M. *$P < 0.05$ *versus* NT; †$P < 0.05$ *versus* saline; and ‡$P < 0.05$ *versus* SA + Ang II.

only of Mas but also of AT_1 and/or AT_2 receptors (Table 1). More work is definitely necessary to dissect the precise mechanisms of these heterologous regulations. Parallel to the immunohistochemistry experiments, receptor autoradiography was also performed for AT_1 and AT_2 subtypes, as well as immunofluorescence for Mas expression. As summarized in Fig. 2, we confirmed that ACE2 overexpression resulted in downregulation of the AT_1 receptor and upregulation of both Mas and AT_2 receptor subtypes in various brain regions of the syn-hACE2 mouse model.

In summary, using adenovirus and mouse genetic models, we have shown that overexpression of ACE2 would not only promote the conversion of the vasoconstrictor, Ang II, into the vasodilator, Ang-(1–7), but would also modulate the expression of their various receptors to the detriment of hypertension. Moreover, increased expression of constitutive NOS isoforms and phosphorylation of eNOS were associated with the receptor modulation and may have promoted enhanced NO release, which would also contribute to the improvement of baroreflex and autonomic functions and ultimately to the buffering of hypertension in this model (Fig. 2).

Conclusion

As an important member of the RAS, ACE2 has been reported to participate in the regulation of blood pressure and cardiovascular function in the brain and the periphery. In this short review of our recent work, we provide evidence that modulation of ACE2 expression in the CNS may play an important role in protecting against the development of neurogenic hypertension through regulation of baroreflex and autonomic function. The molecular mechanisms by which this protective effect occurs seem to include the regulation of expression of angiotensin receptors. ACE2 appears to be able to adjust the AT_2/AT_1 and Mas/AT_1 ratios in a way that opposes the development of hypertension. We also showed that NO signalling pathways are affected by ACE2 overexpression in the CNS and might participate in the overall reduction of the neurogenic hypertension in syn-hACE2 mice. In conclusion, ACE2 plays a regulatory role in the central regulation of blood pressure and cardiovascular function and could become an attractive target for the treatment of hypertension and other cardiovascular diseases resulting from an overactive RAS.

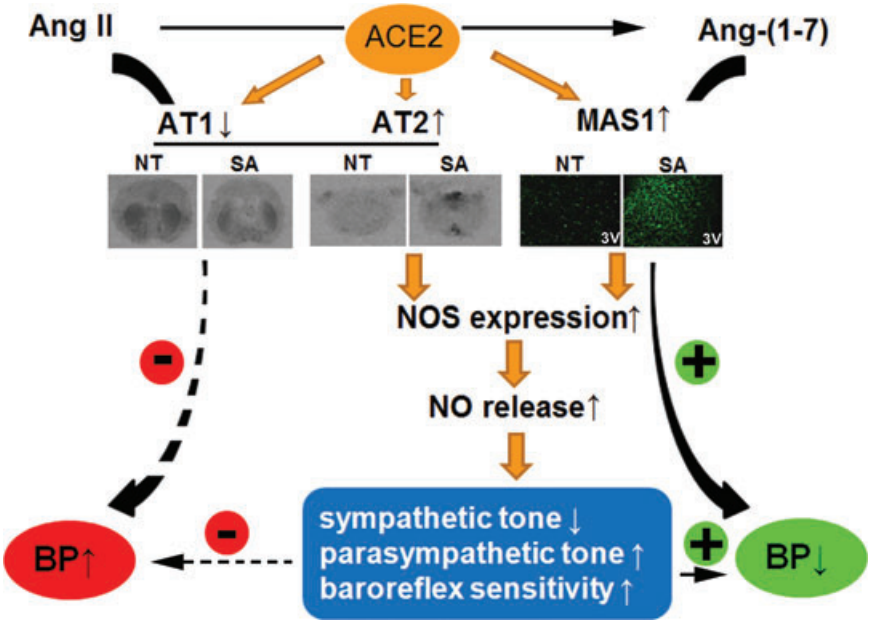

Figure 2. Angiotensin-converting enzyme type 2 (ACE2) and blood pressure regulation
Overexpression of ACE2 in the central nervous system regulates expression of AT_1, AT_2 and Mas receptors and activates the nitric oxide synthase signalling pathway, leading to the modulation of baroreflex sensitivity, sympathetic and parasympathetic tone and eventually resulting in the decrease of neurogenic hypertension. (+) indicates a reinforcement and (−) indicates an inhibition of the pathways. Abbreviations: NT, non-transgenic mice; and SA, syn-hACE2 transgenic mice.

References

Bader M & Ganten D (2008). Update on tissue renin–angiotensin systems. *J Mol Med* **86**, 615–621.

Davisson RL (2003). Physiological genomic analysis of the brain renin–angiotensin system. *Am J Physiol Regul Integr Comp Physiol* **285**, R498–R511.

Diez-Freire C, Vazquez J, Correa de Adjounian MF, Ferrari MFR, Yuan L, Silver X, Torres R & Raizada MK (2006). ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. *Physiol Genomics* **27**, 12–19.

Faure S, Bureau A, Oudart N, Javellaud J, Fournier A & Achard J-M (2008). Protective effect of candesartan in experimental ischemic stroke in the rat mediated by AT_2 and AT_4 receptors. *J Hypertens* **26**, 2008–2015.

Feng Y, Yue X, Xia H, Bindom SM, Hickman PJ, Filipeanu CM, Wu G & Lazartigues E (2008). Angiotensin-converting enzyme 2 overexpression in the subfornical organ prevents the angiotensin II-mediated pressor and drinking responses and is associated with angiotensin II type 1 receptor downregulation. *Circ Res* **102**, 729–736.

Goldstein DS (1983). Plasma catecholamines and essential hypertension. An analytical review. *Hypertension* **5**, 86–99.

Grassi G & Mancia G (2004). Neurogenic hypertension: is the enigma of its origin near the solution? *Hypertension* **43**, 154–155.

Johnson AK & Thunhorst RL (1997). The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Front Neuroendocrinol* **18**, 292–353.

Julius S & Majahalme S (2000). The changing face of sympathetic overactivity in hypertension. *Ann Med* **32**, 365–370.

Lavoie JL & Sigmund CD (2003). Minireview: overview of the renin-angiotensin system—an endocrine and paracrine system. *Endocrinology* **144**, 2179–2183.

Lazartigues E, Feng Y & Lavoie JL (2007). The two fACEs of the tissue renin–angiotensin systems: implication in cardiovascular diseases. *Curr Pharm Des* **13**, 1231–1245.

Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE & Sigmund CD (1996). Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. *J Clin Invest* **97**, 1047–1055.

Nagata S, Kato J, Sasaki K, Minamino N, Eto T & Kitamura K (2006). Isolation and identification of proangiotensin-12, a possible component of the renin–angiotensin system. *Biochem Biophys Res Commun* **350**, 1026–1031.

Paul M, Poyan Mehr A & Kreutz R (2006). Physiology of local renin-angiotensin systems. *Physiol Rev* **86**, 747–803.

Rentzsch B, Iliescu R, Todiras M, Popova E, Baltatu O, Santos R & Bader M (2007). Transgenic ACE2 overexpression in vascular smooth muscle of SHR-SP rats reduces blood pressure and improves endothelial function. *Hypertension* **50**, e89.

Sakai K, Hirooka Y, Shigematsu H, Kishi T, Ito K, Shimokawa H, Takeshita A & Sunagawa K (2005). Overexpression of eNOS in brain stem reduces enhanced sympathetic drive in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol* **289**, H2159–H2166.

Sakima A, Averill DB, Gallagher PE, Kasper SO, Tommasi EN, Ferrario CM & Diz DI (2005). Impaired heart rate baroreflex in older rats: role of endogenous angiotensin-(1–7) at the nucleus tractus solitarii. *Hypertension* **46**, 333–340.

Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL & Touyz RM (2007). Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. *Hypertension* **49**, 185–192.

Schiavone MT, Santos RAS, Brosnihan KB, Khosla MC & Ferrario CM (1988). Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1–7) heptapeptide. *Proc Natl Acad Sci U S A* **85**, 4095–4098.

Sosa-Canache B, Cierco M, Gutierrez CI & Israel A (2000). Role of bradykinins and nitric oxide in the AT_2 receptor-mediated hypotension. *J Hum Hypertens* **14**(Suppl 1), S40–S46.

Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G & Turner AJ (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* **275** 33238–33243.

Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A & Tummino P (2002). Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem* **277**, 14838–14843.

Xia H, Feng Y, Obr TD, Hickman PJ & Lazartigues E (2009). Angiotensin II type 1 receptor-mediated reduction of angiotensin-converting enzyme 2 activity in the brain impairs baroreflex function in hypertensive mice. *Hypertension* **53**, 210–216.

Xu P, Costa-Goncalves AC, Todiras M, Rabelo LA, Sampaio WO, Moura MM, Santos SS, Luft FC, Bader M, Gross V, Alenina N & Santos RAS (2008). Endothelial dysfunction and elevated blood pressure in *Mas* gene-deleted mice. *Hypertension* **51**, 574–580.

Yamazato M, Yamazato Y, Sun C, Diez-Freire C & Raizada MK (2007). Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. *Hypertension* **49**, 926–931.

Yang R, Smolders I, De Bundel D, Fouyn R, Halberg M, Demaegdt H, Vanderheyden P & Dupont AG (2008). Brain and peripheral angiotensin II type 1 receptors mediate renal vasoconstrictor and blood pressure responses to angiotensin IV in the rat. *J Hypertens* **26**, 998–1007.

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR & Davisson RL (2002). Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* **91**, 1038–1045.

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